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adding a set of oligonucleotide primer pairs to said sample, wherein said set of oligonucleotide primers comprises at least one oligonucleotide primer pair capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic *E. coli*, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains;

subjecting said sample and said set of primer pairs to an amplification process to produce amplified product; and

detecting the presence of amplified product.

22. The method according to claim 21, wherein the set of oligonucleotide primer pairs comprises primer pairs selected from the group consisting of:

a primer pair that hybridizes to a gene encoding heat labile toxin, or a gene encoding heat stable toxin for amplification of a DNA sequence characteristic for enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stable toxin for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid for amplification a DNA sequence contained in enteroinvasive *E. coli*;

a primer pair that hybridizes to the EAF plasmid, or the eae gene for amplification of a DNA sequence characteristic for enteropathogenic *E. coli*; and

a primer pair that hybridizes to the genes encoding shiga-like toxin stxI or stxII for amplification of a DNA sequence characteristic for enterohemorrhagic *E. coli*.

23. The method according to claim 22, wherein the oligonucleotide primer pair that hybridizes to the gene encoding heat labile toxin characteristic for enterotoxigenic *E. coli* is

LT-I: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO.: 1) and  
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO.: 2);

the oligonucleotide primer pair that hybridizes to the gene encoding heat stable toxin characteristic for enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO.: 3) and  
ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO.: 4);

the oligonucleotide primer pair that hybridizes to the gene encoding heat stable toxin characteristic for enteroaggregative *E. coli* is

EASTI-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO.: 5) and  
EASTI-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO.: 6);

the oligonucleotide primer pair which hybridizes to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO.: 7) and  
EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO.: 8);

the oligonucleotide primer pair which hybridizes to the inv-plasmid is

EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO.: 9) and  
EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO.: 10);

the oligonucleotide primer pair which hybridizes to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO.: 11) and  
EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO.: 12);

the oligonucleotide primer pair which hybridizes to the eae gene is

EPeh-1: 5' CCC GCA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO.: 13) and  
EPeh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO.: 14);

the oligonucleotide primer pair which hybridizes to the gene encoding shiga-like toxin SttI is

SttI-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO.: 15) and

SltII-2 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO.: 16); and  
the oligonucleotide primer pair which hybridizes to the gene encoding shiga-like  
toxin SltII is

SltII-1: 5' ATG AAG AAG ATR WTT RTD GCR CYT TTA TTY G 3' (SEQ ID NO.:  
17) and

SltII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3' (SEQ  
ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

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24. The method according to claim 21, further comprising an oligonucleotide probe  
capable of hybridizing to a DNA sequence of a virulence factor/toxin gene characteristic for one  
of the subgroups of pathogenic *E. coli*, said subgroups comprising enterotoxigenic,  
enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and  
wherein said oligonucleotide probe is labeled at the 5' end with a fluorescent dye and at the 3'  
end with a fluorescent quencher dye and is susceptible to 5'-3' exonuclease degradation by a  
polymerase, and wherein said amplification process uses a polymerase having 5'-3' exonuclease  
degradation activity.

25 The method according to claim 24 wherein the labeled oligonucleotide probe is  
specific for the respective virulence factor/toxin gene to be detected.

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26. The method according to claim 25 wherein  
the labeled oligonucleotide probe is specific for the detection of heat labile toxin gene  
characteristic for enterotoxigenic *E. Coli*;

the labeled oligonucleotide probe is specific for the detection of heat stabile toxin gene  
characteristic for enterotoxigenic *E. Coli*;

the labeled oligonucleotide probe is specific for the detection of heat stabile toxin gene  
characteristic for enteroaggregative *E. Coli*;

the labeled oligonucleotide probe is specific for the detection of pCVD432 plasmid;

the labeled oligonucleotide probe is specific for the detection of the inv-plasmid;

the labeled oligonucleotide probe is specific for the detection of the EAF-plasmid;

the labeled oligonucleotide probe is specific for the detection of the eae gene;

the labeled oligonucleotide probe is specific for the detection of shiga-like toxin StII gene; and

the labeled oligonucleotide probe is specific for the detection of shiga-like toxin StII gene.

27. The method according to claim 26 wherein the labeled oligonucleotide probe for the detection of heat labile toxin gene characteristic for enterotoxigenic *E. coli* is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG 3' (SEQ ID NO.: 19)

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enterotoxigenic *E. coli* is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3' (SEQ ID NO.: 20);

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enteroaggregative *E. coli* is

5' ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3' (SEQ ID NO.: 21);

the labeled oligonucleotide probe for the detection of pCVD432 plasmid is

3' CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3' (SEQ ID NO.: 22);

the labeled oligonucleotide probe for the detection of the inv-plasmid is

5' CAA AAA CAG AAG AAC CTA TGT CTA CCT 3' (SEQ ID NO.: 23)

the labeled oligonucleotide probe for the detection of the EAF-plasmid is

5' CTT GGA GTG ATC GAA CGG GAT CCA AAT 3' (SEQ ID NO.: 24);

the labeled oligonucleotide probe for the detection of the eae gene is

5' TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3' (SEQ ID NO.: 25);

the labeled oligonucleotide probe for the detection of shiga-like toxin SttI gene is

5' TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3' (SEQ ID NO.: 26);

the labeled oligonucleotide probe for the detection of shiga-like toxin SttII gene is

5' CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3' (SEQ ID NO.: 27).

28. The method according to claim 24 wherein the fluorescent reporter dye is 6-carboxy-fluorescein, tetrachloro-6-carboxy-fluorescein, or hexachloro-6-carboxy-fluorescein, and the fluorescent quencher dye is 6-carboxytetramethyl-rhodamine.

29. The method according to claim 21 wherein the amplification process comprises 35 PCR cycles at a MgCl<sub>2</sub> concentration of 5.2 mmol, an annealing temperature of 55 °C and an extension temperature of 65 °C.

30. A set of oligonucleotide primer pairs useful for polymerase chain reaction (PCR) amplification of DNA of pathogenic enterobacteria allowing detection and differentiation of pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of the pathogenic *E. coli* strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one oligonucleotide primer pair is included in said set of oligonucleotide primer pairs.

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31. The set of primer pairs according to claim 30 comprising

a primer pair that hybridizes to a gene encoding heat labile toxin, or to a gene encoding heat stabile toxin of enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stabile toxin of enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid of enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid of enteroinvasive *E. coli*;

a primer pair that hybridizes to the EAF plasmid, or the eae gene of enteropathogenic *E. coli*; and

a primer pair that hybridizes to the gene encoding shiga-like toxin stI or stII of enterohemorrhagic *E. coli*.

32. The set of primer pairs according to claim 31 wherein

the primer pair which hybridizes to the gene encoding heat labile toxin of enterotoxigenic *E. coli* is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO.: 1) and  
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO.: 2);

the primer pair which hybridizes to the gene encoding heat stabile toxin of enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO.: 3) and  
ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO.: 4);

the primer pair which hybridizes to the gene encoding heat stabile toxin of enteroaggregative *E. coli* is

EASTI-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO.: 5) and

EASTI-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO.: 6);

the primer pair which hybridizes to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO.: 7) and

EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO.: 8);

the primer pair which hybridizes to the inv-plasmid is

EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO.: 9) and

EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO.: 10)

the primer pair which hybridizes to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO.: 11) and

EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO.: 12);

the primer pair which hybridizes to the eae gene is

EPeh-1: 5' CCC GGA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO.: 13) and

EPeh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO.: 14);

the primer pair which hybridizes to the shiga-like toxin sltI gene is

SlitI-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO.: 15) and

SlitI-2: 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO.: 16); and

the primer pair which hybridizes to the shiga-like toxin sltII is

SlitII-1: 5' ATG AAG AAG ATR WTT RTD GCR GYT TTA TTY G 3' (SEQ ID NO.: 17)

and

SlitII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3' (SEQ ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

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33. A set of labeled oligonucleotide probes useful for detection and differentiation of pathogenic enterobacteria in a sample by Real Time-PCR, each probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic *E. coli* strains comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for detection and differentiation of each subgroup at least one probe is included in the set of oligonucleotide probes.

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34. The set of probes according to claim 33 comprising

- a labeled oligonucleotide probe specific for the detection of heat labile toxin gene characteristic for enterotoxigenic *E. Coli*;
- a labeled oligonucleotide probe specific for the detection of heat stabile toxin gene characteristic for enterotoxigenic *E. Coli*;
- a labeled oligonucleotide probe specific for the detection of heat stabile toxin gene characteristic for enteroaggregative *E. Coli*;
- a labeled oligonucleotide probe specific for the detection of pCVD432 plasmid;
- a labeled oligonucleotide probe specific for the detection of the *inv*-plasmid;
- a labeled oligonucleotide probe specific for the detection of the EAF-plasmid;
- a labeled oligonucleotide probe specific for the detection of the *eae* gene;
- a labeled oligonucleotide probe specific for the detection of shiga-like toxin StI gene;

and

- a labeled oligonucleotide probe specific for the detection of shiga-like toxin StII gene.

35. The set of probes according to claim 34 wherein

the labeled oligonucleotide probe for the detection of heat labile toxin gene characteristic for enterotoxigenic *E. coli* is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG 3' (SEQ ID NO.: 19);

the labeled oligonucleotide probe for the detection of heat stabile toxin gene characteristic for enterotoxigenic *E. coli* is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3' (SEQ ID NO.: 20);



the labeled oligonucleotide probe for the detection of heat stable toxin gene characteristic for enteroaggregative *E. coli* is

5' ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3' (SEQ ID NO.: 21);

the labeled oligonucleotide probe for the detection of pCVD432 plasmid is

5' CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3' (SEQ ID NO.: 22);

the labeled oligonucleotide probe for the detection of the inv-plasmid is

5' CAA AAA CAG AAG AAC CTA TGT CTA CCT 3' (SEQ ID NO.: 23)

the labeled oligonucleotide probe for the detection of the EAF-plasmid is

5' CTT GGA GTG ATC GAA CGG GAT CCA AAT 3' (SEQ ID NO.: 24)

the labeled oligonucleotide probe for the detection of the eae gene is

5' TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3' (SEQ ID NO.: 25)

the labeled oligonucleotide probe for the detection of shiga-like toxin SltI gene is

5' TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3' (SEQ ID NO.: 26);

the labeled oligonucleotide probe for the detection of shiga-like toxin SltII gene is

5' CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3' (SEQ ID NO.: 27).

36. A set of oligonucleotide primer pairs and a set of oligonucleotide primer probes useful for diagnosing an enterobacteria infection in samples derived from a living animal body including a human, by Real time PCR method, wherein said set of oligonucleotide primer pairs allows detection and differentiation of pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of the pathogenic *E. coli* strains said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one oligonucleotide primer pair is included in said set of oligonucleotide probes and a set of oligonucleotide probes, wherein said set of oligonucleotide probes detect virulence factor/toxin genes characteristic of one of the

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subgroups of pathogenic *E. coli* strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains by real time PCR

37. The method of claim 21, wherein said method is used to diagnose an enterobacteria infection in a sample derived from a living animal body.

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38. The method of claim 27, wherein said sample is derived from a human.

39. The method of claim 21, wherein said method is used to detect enterobacteria contamination of a consumable.

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40. The method of claim 29, wherein said consumable is selected from the group consisting of meat, milk or vegetable.

#### REMARKS

Claims 1-20 are pending in this application and have been canceled without prejudice or disclaimer. Claims 21-40 are newly added. Support for the newly added claims may be found throughout the specification. For example, support for claim 21 may be found at page 4, lines 13-16 and page 5, lines 15-20; for claims 22 and 31 at page 6, lines 8-30, page 7, lines 1-6 and page 11, lines 8-26; for claims 24 and 33 at page 7, lines 10-35, page 8, lines 1-30, page 12, lines 1-30 and page 13, lines 1-23; for claim 25 at page 4, lines 14-16; for claims 26, 27, 34 and 35 at page 9, lines 15-30, page 10, lines 15-30, page 14, lines 5-31 and page 15, lines 1-8; for claim 28 at the bridging paragraph on pages 10-11; for claim 29 at page 11, lines 3-5, for claim 30 at page 11, lines 8-26, page 14, lines 6-31 and page 15, lines 1-2; for claim 36 at page 11, lines 8-26, page 14, lines 6-31 and page 15, lines 1-2 and for claims 37-40 at the bridging paragraph at pages 19-20 and page 15, lines 6-8. Thus, no new matter has been added. Reconsideration of the application in view of the following remarks is respectfully requested.